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I, the below-named translator, hereby declare:

(1) That my name, mailing address and citizenship are as stated below;

(2) That I am knowledgeable in the English language and in the Korean language in which Korean Patent Application No. 2003-987 was filed on January 8, 2003; and

(3) That I have translated said Korean Patent Application No. 2003-987 into English, which English text is attached hereto, and believe that said translation is a true and complete translation of the aforementioned Korean patent application.

October 5, 2006

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FILING DETAILS OF THE PATENT APPLICATION

Application Number: 10-2003-0000987
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[TITLE OF THE INVENTION]
Beta-catenin oligonucleotide microchip and method for detecting beta-catenin mutations employing same

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The above application is filed in accordance with Article 42 of Korean Patent Law, and the

request for the examination of the above application is filed in accordance with Article 60 of Korean Patent Law.

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SPECIFICATION

[ABSTRACT]

The present invention relates to a β-catenin oligonucleotide microchip for detecting mutation in the mutational hot spot areas of β-catenin gene, a manufacturing process thereof and a method for detecting the β-catenin mutation employing same, wherein specific oligonucleotides are designed to detect various missense mutations and in-frame deletion at the mutational hot spots of β-catenin gene. Since the β-catenin oligo chip of the present invention employs the selectively designed oligonucleotides specific for the mutational hot spot areas of β-catenin gene, it provides improved accuracy and efficiency in detecting β-catenin gene mutation, and therefore, can be used in studies to detect β-catenin mutations and unravel the signal transduction mechanism and tumorigenesis related to β-catenin gene.

[FIGURE OF THE DRAWINGS]

Fig. 1

[TITLE OF THE INVENTION]

BETA-CATENIN OLIGONUCLEOTIDE MICROCHIP AND METHOD FOR DETECTING BETA-CATENIN MUTATIONS EMPLOYING SAME

[BRIEF DESCRIPTION OF THE DRQWINGS]

Fig. 1 shows the result of detecting β-catenin mutation in colon cancer tissue using the inventive β-catenin oligonucleotide microchip;

Fig. 2 shows the direct sequencing result of colon cancer tissue which has a β-catenin mutation confirmed by the inventive β-catenin oligonucleotide microchip; and

Fig. 3 shows the PCR-SSCP analysis result of colon cancer tissue which has a β-catenin mutation confirmed by the inventive β-catenin oligonucleotide microchip.

[DETAILED DESCRPTION OF THE INVENTION]

[OBJTECT OF THE INVENTION]

[FIELD OF THE INVENTION AND BACKGROUND OF THE INVENTION]

The present invention relates to a β-catenin oligonucleotide microchip for detecting mutations in the mutational hot spot areas of β-catenin gene, a manufacturing process thereof and a method for detecting β-catenin mutations employing same.

β-Catenin, which functions as a downstream transcriptional activator in the Wnt signaling pathway, is a submembrane component of the cadherin-mediated cell-cell adhesion system (Abraham, S. C. et al., *Am. J. Pathol.* 158:1005-1010, 2001; Abraham, S. C. et al., *Am. J. Pathol.* 158:1073-1078, 2001). APC (adenomatus polyposis coli) tumor suppressor protein, along with GSK-3β (glycogen synthase kinase-3β), promotes the phosphorylation of the serine/thereonine residues in exon 3 of the β-catenin gene (Abraham, S. C. et al., *Am. J. Pathol.* 158:1073-1078, 2001). Mutation of the *APC* gene or the β-catenin gene was found to result in the accumulation of β-catenin protein and the loss of β-catenin regulatory activity (Abraham, S. C. et al., *Am. J. Pathol.* 158:1073-1078, 2001). The

majority of β-catenin mutations have been reported at specific GSK-3β phosphorylation sites, i.e., Ser-33, Ser-37, Thr-41, Ser-45, and other residues (Asp-32 and Gly-34) in many human cancers, including endometrial, gastric, ovarian, hepatoblastomas, and colorectal cancers (Saegusa, M. and Okayasu, I. *J. Pathol.* 194:59-67, 2001). In colorectal cancers, various frequencies of the β-catenin mutations have been reported, ranging from 0 to 16% (Nilbert, M. and Rambech, E. *Cancer Genet. Cytogenet.* 128:43-45, 2001; Mirabelli-Primdahl, L. et al., *Cancer Res.* 59:3346-51, 1999). Most β-catenin mutations are restricted at some codons in exon 3, and substitution mutations causing amino acid changes predominate in the β-catenin gene (Devereux, T. R. et al., *Mol. Carcinog.* 31:68-73, 2001; Udatsu, Y. et al., *Pediatr. Surg. Int.* 17:508-512, 2001; Koch, A. et al., *Cancer Res.* 59:269-273, 1999; de La Coste, A. et al., *Proc. Natl. Acad. Sci. USA* 95:8847-8851, 1998).

Although it seems easy to detect β-catenin gene mutations using conventional methods, such as PCR-SSCP (single strand conformation polymorphism) and direct sequencing, technical problems associated with the low sensitivity of such β-catenin mutation detection methods have been reported (Abraham, S. C. et al., *Am. J. Pathol.* 158:1005-1010, 2001). Thus, there has been a need to develop a more reliable and faster mutation detection technique for β-catenin gene which can be used for various cancer studies, e.g., elucidation of the Wnt signaling related mechanism.

Studies have suggested that the high frequency MSI (microsatellite instability-H, MSI-H) colorectal cancer is not linked to APC mutations (Mirabelli-Primdahl, L. et al., *Cancer Res.* 59:3346-51, 1999), and that β-catenin gene mutations are mainly induced in MSI-H colorectal carcinomas (Mirabelli-Primdahl, L. et al., *Cancer Res.* 59:3346-51, 1999; Shitoh, K. et al., *Genes Chromosomes Cancer* 30:32-37, 2001). It has been also reported that in case of proximal colorectal carcinomas, MSI-H colorectal carcinomas are involved in the location of tumors (Mirabelli-Primdahl, L. et al., *Cancer Res.* 59:3346-51, 1999; Traverso, G. et al., *Lancet.* 359:403-404, 2002). However, it has been still unknown the relationship between β-catenin mutations and proximal colorectal carcinomas.

Traverso et al. used MSI in the stool as a marker for the diagnosis of proximal colon cancers in stools (Traverso, G. et al., *Lancet.* 359:403-404, 2002), and several other markers, such as *APC*, *p53*, long DNA and *K-ras*, have been also used for colorectal cancer diagnosis using fecal DNA

(Ahlquist, D. A. et al., *Gastroenterology* 119:1219-1227, 2000; Dong S. M. et al., *J. Natl. Cancer. Inst.* 93:858-865, 2001).

The fact that β-catenin mutations are prone to occur in proximal colon cancers suggests β-catenin mutations might be used to diagnose proximal colon cancer. Accordingly, the present inventors have developed a β-catenin oligonucleotide microchip manufactured by fixing oligonucleotides on the surface of a solid matrix using an automatic microarrayer, the oligonucleotides being designed to detect various mutations at mutational hot spot areas of β-catenin gene. The β-catenin oligonucleotide microchip of the present invention can be used in studies to detect β-catenin mutations and to unravel the signal transduction mechanism and tumorigenesis related to β-catenin gene.

[SUMMARY OF THE INVENTION]

Accordingly, an object of the present invention is to provide a β-catenin oligonucleotide microchip which can be used as a fast and reliable genetic diagnostic device for studying the signal transduction mechanism and tumorigenesis related to β-catenin gene.

It is another object of the present invention to provide a method for detecting the β-catenin mutation using the β-catenin oligonucleotide microchip

[DETAILED DESCRIPTION OF THE INVENTION]

In accordance with one aspect of the present invention, there is provided a β-catenin oligonucleotide microchip for detecting β-catenin mutations comprising a plurality of oligonucleotides fixed on the surface of a solid matrix, wherein the oligonucleotides are designed to detect mutations in the mutational hot spots of β-catenin gene.

In accordance with still another aspect of the present invention, there is provided a manufacturing process thereof.

In accordance with still another aspect of the present invention, there is provided a method for detecting β-catenin mutations employing same.

Hereinafter, the present invention is described in detail.

The present invention provides a β-catenin oligonucleotide microchip (hereinafter, referred to as "β-catenin oligo chip") for detecting

β-catenin mutations, which comprises oligonucleotides fixed on the surface of a solid matrix using an automatic microarrayer, wherein the oligonucleotides are capable of detecting various mutations at mutational hot spot areas of β-catenin gene.

First, the oligonucleotides are designed to detect all possible missense mutations and in-frame deletions at 11 codons (codons 29, 31, 32, 33, 34, 35, 37, 38, 41, 45 and 48) in exon 3, mutational hot spots of β-catenin gene.

β-Catenin mutations have been identified in a variety of human malignancies, most of being missense mutations resctricted at hot-spot areas in exon 3. β-Catenin mutations are known to be associated with colorectal cancers with MSI. More than 70% of β-catenin mutations have been reported in colorectal cancers, and about 90% of those at the 11 codons in the hot spot area (codons 29, 31, 32, 33, 34, 35, 37, 38, 41, 45 and 48).

The present invention provides oligonucleotides which can be used to detect all possible mutations at the above mentioned hot spots of β-catenin gene, which occur at a frequency of more than 90% of all cases examined. Therefore, the β-catenin oligo chip of the present invention makes it possible to detect mutation at a confidence level of over 90%. In addition, since the oligonucleotides used in the inventive β-catenin oligo chip are designed to detect all possible missense mutations at the 11 codons, it is capable of detecting any missense mutation at these codons which have not yet been discovered. Further, the inventive β-catenin oligo chip also includes the oligonucleotides for detecting in-frame deletion (3-bp deletion) at each of the hot spot codons. Namely, as the inventive oligonucleotides are specifically designed to detect mutations at the hot spots of β-catenin gene taking the gene characteristics into consideration, the inventive β-catenin oligo chip provides improved accuracy and efficiency in detecting β-catenin gene mutation.

According to one aspect of the present invention, the inventive β-catenin oligo chip has 121 types of oligonucleotides spotted and fixed on the surface of a solid matrix, the oligonucleotides being capable of detecting 99 types of all possible missense mutations, 11 types of in-frame deletions and 11 types of wild types at each of the 11 hot spot codons of β-catenin gene. Each oligonucleotide is spotted 4 times horizontally for increased accuracy of measured signals.

Nine oligonucleotides (M) are designed to cover all possible substitutions at each hot spot codon and one oligonucleotide (W) for the

wild type. Thus, a total of 10 oligonucleotides are designed to detect missense mutations for codons 23, 29, 31, 32, 33, 34, 35, 38, 41 and 48. Further, 11 oligonucleotides (D) are designed to detect in-frame deletions (3-bp deletion) for each hot spot codon. In total, the 121 oligonucleotides cover all substitutions and in-frame deletions in the above 11 codons of exon 3. Specifically, used for codon 29 are 9 types of substituted oligonucleotides obtained by replacing TCT (serine) with ACT (threonine), GCT (alanine), CCT (proline), TAT (tyrosine), TGT (cytosine), TTT (phenylalanine), TCA (serine), TCG (serine) and TCC (serine), respectively, and one deletion oligonucleotide obtained by deleting 3 bp of TCT. Used for codon 31 are 9 types of substituted oligonucleotides obtained by replacing CTG (leucine) with ATG (methionine), TTG (leucine), GTG (valine), CAG (glutamine), CGG (arginine), CCG (proline), CTA (leucine), CTC (leucine) and CTT (leucine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of CTG. Used for codon 32 are 9 types of substituted oligonucleotides obtained by replacing GAC (aspartic acid) with CAC (histidine), TAC (tyrosine), AAC (asparagines), GCC (alanine), GTC (valine), GGC (glycine), GAG (glutamic acid), GAT (aspartic acid) and GAA (glutamic acid), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of GAC. Used for codon 33 are 9 types of substituted oligonucleotides obtained by replacing TCT (serine) with ACT (threonine), GCT (alanine), CCT (proline), TGT (cysteine), TAT (tyrosine), TTT (phenylalanine), TCA (serine), TCG (serine) and TCC (serine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of TCT. Used for codon 34 are 9 types of substituted oligonucleotides obtained by replacing GGA (glycine) with TGA (stop codon), AGA (arginine), CGA (arginine), GTA (valine), GCA (alanine), GAA (glutamic acid), GGT (glycine), GGG (glycine) and GGC (glycine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of GGA. Used for codon 35 are 9 types of substituted oligonucleotides obtained by replacing ATC (isoleucine) with GTC (valine), CTC (leucine), TTC (phenylalanine), ACC (threonine), AGC (serine), AAC (asparagine), ATG (methionine), ATA (isoleucine) and ATT (isoleucine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of ATC. Used for codon 37 are 9 types of substituted oligonucleotides obtained by replacing TCT (serine) with ACT (threonine), CCT (proline), GCT (alanine), TAT (tyrosine), TGT (cysteine), TTT (phenylalanine), TCA (serine), TCG (serine) and TCC (serine), respectively; and one deletion oligonucleotide obtained by deleting

3 bp of ACT. Used for codon 38 are 9 types of oligonucleotides obtained by replacing GGT (glycine) with AGT (serine), CGT (arginine), TGT (cysteine), GAT (aspartic acid), GCT (alanine), GTT (valine), GGA (glycine), GGG (glycine) and GGC (glycine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of GGT. Used for codon 41 are 9 types of substituted oligonucleotides obtained by replacing ACC (threonine) with TCC (serine), GCC (alanine), CCC (proline), AGC (serine), ATC (isoleucine), AAC (asparagine), ACA (threonine), ACT (threonine) and ACG (theronine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of ACC. Used for codon 45 are 9 types of substituted oligonucleotides obtained by replacing TCT (serine) with ACT (threonine), GCT (alanine), CCT (proline), TGT (cysteine), TAT (tyrosine), TTT (phenylalanine), TCA (serine), TCG (serine) and TCC (serine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of ACT. Used for codon 48 are 9 types of substituted oligonucleotides obtained by replacing GGT (glycine) with AGT (serine), TGT (cysteine), CGT (arginine), GAT (aspartic acid), GCT (alanine), GTT (valine), GGA (glycine), GGC (glycine) and GGG (glycine), respectively; and one deletion oligonucleotide obtained by deleting 3 bp of GGT.

One wild type of oligonucleotide (W) is designed for each codon to be directly compared with mutation types and to cover both homozygous and heterozygous mutations. For example, 12 oligonucleotides are spotted for codon 29, one is to detect a normal base sequence and the rest, the mutated base sequences. As a whole, 110 mutant oligonucleotides are designed for the 99 missense mutation types and 11 in-frame deletion types at the 11 hot spot codons, and 11 oligonucleotides, for the wild types and positive controls.

The β-catenin oligo chip of the present invention may be manufactured by fixing as many as 121 oligonucleotides on the surface of a solid matrix using an automatic microarrayer by a process comprising the steps of:

1) mixing each of the oligonucleotides in a micro spotting solution and distributing to a well plate;

2) spotting the oligonucleotide on the surface of a solid matrix using a microarrayer;

3) fixing the oligonucleotides on the solid matrix surface and washing;

4) denaturing the fixed oligonucleotides by soaking the solid matrix

in 95℃ water, and then, treating the solid matrix with a sodium borohydride solution; and

5) washing and drying the solid matrix.

Each of the oligonucleotides used in step (1) preferably has a functional group that can be used to form a stable bond with the solid matrix surface. For example, each oligonucleotide may be linked with a 12 carbon spacer having a 5' amino modification, e.g., H_2N-$(CH_2)_{12}$-oligonucleotide. This amine group undergoes Schiff's base reaction with an aldehyde group on the solid matrix to form a firm bond therebetween. The 12 carbon spacer serves to enhance the hybridization rate by facilitating the contact between the oligonucleotide and a fluorescent dye-labeled target DNA.

The micro spotting solution used in step (1) may contain suitable salts and polymers to facilitate the application of the oligonucleotides on the solid matrix.

The solid matrix used in step (2) may be made of glass; modified silicone; plastic cassette; or polymer such as polycarbonate or a gel thereof. The surface of a solid matrix may be coated with a chemical compound that can serve to bind the oligonucleotide to the matrix substrate. Preferable chemicals that can be used for such coating have functional groups such as aldehyde or amine groups. In one preferred embodiment, the present invention uses a slide glass coated with an aldehyde.

According to one embodiment of steps (1) and (2), a total of 484 oligonucleotides are arranged in a specified manner on a solid matrix using an automatic pin microarrayer. Each oligonucleotide spot is preferably of circular shape with a diameter ranging from 100 to 500 ㎛. A preferable example of the solid matrix is a 3.7 ㎝×7.6 ㎝ slide glass, which can accommodate approximately 100 to 10,000 spots per chip. Preferably, a total of 484 oligonucleotide spots, each of 130 ㎛ diameter, may be arranged in multiple columns and rows at intervals of 200 to 800 ㎛.

In step (3), the oligonucleotides are fixed on the solid matrix surface by way of forming covalent bonds between the amine groups of the oligonucleotide and the aldehyde groups of the solid matrix via Schiff's base reaction. Free unreacted oligonucleotides are removed by washing the solid matrix with SDS, SSC, SSPE, etc.

In step (4), the fixed oligonucleotides are denatured by soaking the microchip in boiling water at 95℃ to prevent the oligonucleotides from being aggregated, unreacted aldehyde groups remaining on the solid matrix

are reduced and inactivated by 0.09 M sodium borohydride treatment.

The β-catenin oligo chip of the present invention manufactured by the above process may be advantageously used to detect gene mutation, and this inventive method is much simpler and more economical than any of the conventional gene mutation detection methods: It takes several days to months on the average when the presence of gene mutation is examined using such conventional methods as SSCP (single strand conformation polymorphism), PTT (protein truncation test), RFLP (restriction fragment length polymorphism), cloning, direct sequencing, etc. However, analysis of a DNA sample for β-catenin gene mutation takes less than 10 to 11 hours when the inventive β-catenin oligo chip is employed. In addition, the β-catenin oligo chip of the present invention can be manufactured much more simply at a much less production cost than conventional chips. Once the required oligonucleotides are synthesized, it is possible to mass-produce the inventive slides. The amounts of reagents required when the inventive β-catenin oligo chip is used are far less than those required in any of the conventional methods.

The β-catenin oligo chip of the present invention is easy to manufacture and use in a laboratory scale. In case of the existing Affymetrix oligo chip, oligonucleotides are directly synthesized on the surface of a solid matrix by using a photolithography technique. However, the β-catenin oligo chip of the present invention employs a method which previously synthesized oligonucleotides and spots the oligonucleotides on the surface of a solid matrix by using a pin microarrayer. Therefore, the β-catenin oligo chip of the present invention can synthesize oligonucleotides for a low price compared with the existing method requiring a high-level of equipment and technique and high cost, and can be easily manufactured and interpreted in a general laboratory.

Further, the existing Affymetrix chip requires a private hybridization tool and scanner, while since the β-catenin oligo chip of the present invention can be read by using a common DNA chip reader and its hybridization reaction is also carried out by hand without using such special tools, it is possible to conduct a mutation detection experiment using a DNA chip at a low price.

Furthermore, it is possible with the β-catenin oligo chip of the present invention to purify and modify the oligonucleotides. The Affymetrix oligo chip is prepared by directly synthesizing oligonucleotides on the surface of a solid matrix, wherein it is not possible to purify or

modify the oligonucleotides. However, the β-catenin oligo chip of the present invention can easily modify the oligonucleotides, e.g., repeated spotting on a single solid matrix for reducing an experimental error, as well as purify the oligonucleotides in order to improve the quality of oligonucleotides. When considering that the quality of the oligonucleotides determines the over-all accuracy of a chip, the inventive β-catenin oligo chip is capable of satisfying such condition.

The present invention provides a method for detecting the β-catenin mutation employing the β-catenin oligo chip, which comprises the steps of:

1) preparing a fluorescent dye-labeled DNA sample from the blood of a subject patient;

2) reacting the labeled DNA sample with oligonucleotide spots on the β-catenin oligo chip;

3) washing the reacted oligo chip to remove unbound sample DNA;

4) detecting the mode of hybridization of specific oligonucleotide spots using a fluorescence reader; and

5) examining the presence of gene mutation.

In step (1), a DNA sample is prepared by incorporating a fluorescent dye into a blood DNA sample obtained from a subject patient. In the hybridization of fluorescent dye-labeled DNA with certain oligonucleotide spot on the oligo chip, it can be analyzed with a fluorescence reader using an appropriate software. Preferable fluorescent dyes include, but are not limited to, Cy5, Cy3, Texas Red, Fluorescein and Lissamine.

In step (2), the florescent dye-labeled DNA sample prepared in step (1) is mixed with a hybridization solution and transferred to each of the oligonucleotide. The hybridization reaction is performed in a 45~60℃ incubator saturated with water vapor for 3~9 hours.

Then, the oligo chip is washed to remove unbound sample DNA and dried (step 3), and the resulting fluorescence is analyzed with a fluorescence reader using an appropriate software (step 4).

In step (5), setting a maximum value at 99% reliable range as a threshold value, any signal showing a fluorescence level higher than the threshold is regarded positive for the presence of mutation.

The β-catenin oligo chip of the present invention can be effectively used to diagnose such cancer as colorectal carcinomas, endometrial cancer, stomach cancer, ovary cancer, hepatoblastoma cancer, etc. Since β-catenin gene function as a downstream transcriptional activator in the Wnt signaling pathway, the inventive β-catenin oligo chip can be used as an effective

diagnostic tool for the study of signal transduction mechanism and tumorigenesis related to β-catenin gene.

The present invention investigated 74 colorectal carcinomas and 31 colorectal cancer cell lines for the presence of β-catenin mutations (see Fig. 1). All 5 β-catenin mutations were identified in proximal colon cancers (N=34), but β-catenin mutations were absent in 40 distal colorectal cancers. Four out of the 5 β-catenin mutations were point mutations at codons 32, 41 and 45, and the remaining one was in-frame deletion (3 bp deletion) at codon 45. In 31 colorectal cancer cell lines, 4 β-catenin mutations were identified. Three of these 4 mutations occurred at codon 45, and the remaining one occurred at codon 41.

In total 9 mutations were identified in the 74 colorectal carcinomas and 31 colorectal cancer cell lines. Six of the 9 mutations were found at codon 45 and 2 were at codon 41. Of the 6 mutations at codon 45, 4 were the identical missense mutations (TCT→TTT, Ser→Phe; in samples 395, 400, SNU-1047 and LSI17T) and 2 were the same in-frame deletion in samples 396 and HCT116. Codons 41 and 45 are known as GSK-3β phosphorylation sites and mutations at these sites might cause nuclear β-catenin accumulation (Saegusa, M. and Okayasu, I. *J. Pathol.* 194:59-67, 2001).

The remaining β-catenin mutation occurred at codon 32 in colon tissue 207. It has been proposed that codon 32 is important for β-catenin ubiquitination and proteasome-dependent degradation (Tong, J. H. et al., *Cancer Lett.* 163:125-130, 1999). Mutations at codon 32 might influence serine 33 accessibility by GSK-3β kinase, thus preventing its phosphorylation (Koch, A. et al., *Cancer Res.* 59:269-273, 1999). It has been reported that specific codon 45 mutation (Ser45Phe) was frequent in colorectal carcinomas, and that codon 41 mutations, which predominate in hepatoblastomas, are rare in colorectal carcinomas (Koch, A. et al., *Cancer Res.* 59:269-273, 1999).

In the present invention, three of the 5 β-catenin mutations from colorectal cancers and three of the 4 β-catenin mutations from cell lines were identified at codon 45, and two of the 6 mutations at codon 45 were in-frame deletions. The in-frame deletion at codon 45 was previously reported in a colorectal cancer cell line and in colorectal carcinomas, but not in other types of cancer (Ilyas, M. et al., *Proc. Natl. Acad. Sci. USA* 94:10330-10334, 1997; Muller, O. et al., *Genes Chromosomes Cancer* 22:37-41, 1998). The in-frame deletion at codon 45 may result in the loss of highly conserved

serine residues in a region of the protein that serves as a target for the enzyme GSK-3β (Ilyas, M. et al., *Proc. Natl. Acad. Sci. USA* 94:10330-10334, 1997). These results indicate that codon 45 mutations, including the in-frame deletion, are common in colorectal carcinomas but are not common in other types of cancer.

Mutational analysis of the β-catenin gene was performed using the oligonucleotide microarray. As the result of mutational analysis using the inventive β-catenin oligo chip, the 9 β-catenin mutation positive samples in a total of 60 samples were detected. The present inventors compared the 9 β-catenin mutations detected by the β-catenin oligo chip with several techniques, e.g., PCR-SSCP, DHPLC, direct sequencing, cloning-sequencing. Automatic direct sequencing, which has been widely used for mutational analysis was not capable of clearly detecting 2 of the 9 β-catenin mutations (see Fig, 2), and PCR-SSCP also missed one β-catenin mutation (see Fig. 3). These results might have been caused by excessive wild-type DNA in cancer tissues or by the low sensitivity of these two methods.

In the MSI study using the BAT-26 marker, the present invention confirms that MSI is intimately associated with proximal colon cancer, which agrees with previous reports ($p<0.01$) (Mirabelli-Primdahl, L. et al., *Cancer Res.* 59:3346-51, 1999; Traverso, G. et al., *Lancet.* 359:403-404, 2002). MSI was shown in 10 of 34 proximal colon carcinomas (29%), but in only 2 of 40 distal colorectal carcinomas (5%). In terms of the correlation between MSI and β-catenin mutations, β-catenin mutations were detected in 5 of the 12 (42%) colorectal carcinomas with MSI, but none of the 62 (0%) MSS colorectal carcinomas were found to harbor β-catenin mutations. All 5 β-catenin mutations detected in colon carcinomas with MSI were found in proximal colon cancers. These results confirm that MSI is involved in β-catenin mutations and demonstrate that β-catenin mutations are directly associated with proximal colon cancer.

It has been previously suggested that β-catenin mutations account for approximately are half of colorectal cancers with intact APC (Sparks, A. B. et al., *Cancer Res.* 59998:1130-1134, 1998). In the present invention, only one colorectal cancer cell line (SNU-1047) of the 9 samples with β-catenin mutations had *APC* mutations in the MCR. Recently, several groups have tried to diagnose colorectal cancers by using molecular markers such as *APC*, *p53*, long DNA, *K-ras*, etc (Traverso, G. et al., *Lancet.* 359:403-404, 2002; Ahlquist, D. A. et al., *Gastroenterology* 119:1219-1227, 2000; Dong, S. M. et al., *J. Natl. Cancer Inst.* 93:858-865, 2001). Three of

5 markers including MSI have been used for colorectal cancer diagnosis using fecal DNA (Traverso, G. et al., *Lancet*. 359:403-404, 2002; Ahlquist, D.A. et al., *Gastroenterology* 119:1219-1227, 2000; Dong, S. et al., *J. Natl. Cancer Inst*. 93: 858-865, 2001). In addition, MSI has been used for the diagnosis of proximal colon cancers, which is difficult to detect because, among colorectal cancers, they are located furthest from the anus (Traverso, G. et al., *Lancet*. 359:403-404, 2002). β-Catenin may be a diagnostic marker for proximal colon cancer if β-catenin mutations correlate with the tumor's location in the proximal colon. The results of the present invention show MSI in 29% and β-catenin mutations in 15% of proximal colon cancers, respectively. Although all samples with β-catenin mutations exhibited MSI, β-catenin, alone or with MSI, may be used for the diagnosis of proximal colon cancer. Practically, such a system should not only be highly automated but also be usable as a high throughout diagnostic tool, especially if the substrate used in fecal DNA.

The following Examples and Test Examples are intended to further illustrate the present invention without limiting its scope.

<Example 1> Detection of β-catenin mutations using β-catenin oligo chip

(Step 1) Manufacture of β-catenin oligo chip

A total of 110 oligonucleotides were designed to cover all substitutions and in-frame deletions at 11 mutational hot spot codons of β-catenin gene as described in Tables 1a to 1f (Metabion, Germany).

Oligonucleotides having missense mutation at one of the hot spot codons are: the oligonucleotides described in SEQ ID Nos. 2 to 10, at codon 29; the oligonucleotides described in SEQ ID Nos. 13 to 21, at codon 31; the oligonucleotides described in SEQ ID Nos. 24 to 32, at codon 32; the oligonucleotides described in SEQ ID Nos. 35 to 43, at codon 33; the oligonucleotides described in SEQ ID Nos. 46 to 54, at codon 34; the oligonucleotides described in SEQ ID Nos. 57 to 65, at codon 35; the oligonucleotides described in SEQ ID Nos. 68 to 76, at codon 37; the oligonucleotides described in SEQ ID Nos. 79 to 87, at codon 38; the oligonucleotides described in SEQ ID Nos. 90 and 98, at codon 41; the oligonucleotides described in SEQ ID Nos. 101 and 109, at codon 45; and the oligonucleotides described in SEQ ID Nos. 112 and 120, at codon 48.

Further, oligonucleotides having in-frame deletion at one of the hot spot codons are: the oligonucleotide described in SEQ ID No. 11, at codon 29; the oligonucleotide described in SEQ ID No. 22, at codon 31; the oligonucleotide described in SEQ ID No. 33, at codon 32; the oligonucleotide described in SEQ ID No. 44, at codon 33; the oligonucleotide described in SEQ ID No. 55, at codon 34; the oligonucleotide described in SEQ ID No. 66, at codon 35; the oligonucleotide described in SEQ ID No. 77, at codon 37; the oligonucleotide described in SEQ ID No. 88, at codon 38; the oligonucleotide described in SEQ ID No. 99, at codon 41; the oligonucleotide described in SEQ ID No. 110, at codon 45; and the oligonucleotide described in SEQ ID No. 121, at codon 48. The oligonucleotides described in SEQ ID Nos. 1, 12, 23, 34, 45, 56, 67, 78, 89, 100 and 111are wild types.

Each of all 121 oligonucleotides has a 12-carbon spacer to 5'-terminal modified with an amine residue which can undergo Schiff's base reaction with aldehyde groups and purified by HPLC.

<Table 1a>

SEQ ID No.	Oligonucleotide	Exon	Codon	Sequence
1	29W	3	29	5'-CAGCAACAGTCTTACCTGGAC-3'
2	29M1			5'- GCAGCAACAG**ACT**TACCTGGA-3'
3	29M2			5'- GCAGCAACAG**GCT**TACCTGGA-3'
4	29M3			5'- GCAGCAACAG**CCT**TACCTGGA-3'
5	29M4			5'-CAGCAACAG**TAT**TACCTGGAC-3'
6	29M5			5'-CAGCAACAG**TGT**TACCTGGAC-3'
7	29M6			5'-CAGCAACAG**TTT**TACCTGGAC-3'
8	29M7			5'-AGCAACAG**TCA**TACCTGGACT-3'
9	29M8			5'-AGCAACAG**TCG**TACCTGGACT-3'
10	29M9			5'-AGCAACAG**TCC**TACCTGGACT-3'
11	29D			5'-GGCAGCAACAGTACCTGGACT-3'
12	31W		31	5'-CAGTCTTACCTGGACTCTGGA-3'
13	31M1			5'-ACAGTCTTAC**ATG**GACTCTGG-3'
14	31M2			5'-ACAGTCTTAC**TTG**GACTCTGG-3'
15	31M3			5'-ACAGTCTTAC**GTG**GACTCTGG-3'
16	31M4			5'-CAGTCTTAC**CAG**GACTCTGGA-3'
17	31M5			5'-CAGTCTTAC**CGG**GACTCTGGA-3'

18	31M6			5'-CAGTCTTAC**CCG**GACTCTGGA-3'
19	31M7			5'-AGTCTTAC**CTA**GACTCTGGAA-3'
20	31M8			5'-AGTCTTAC**CTC**GACTCTGGAA-3'
21	31M9			5'-AGTCTTAC**CTT**GACTCTGGAA-3'
22	31D			5'-AACAGTCTTACGACTCTGGAA-3'

<Table 1b>

SEQ ID No.	Oligonucleotide	Exon	Codon	Sequence
23	32W	3	32	5'-TCTTACCTGGACTCTGGAATC-3'
24	32M1			5'-GTCTTACCTG**CAC**TCTGGAAT-3'
25	32M2			5'-GTCTTACCTG**TAC**TCTGGAAT-3'
26	32M3			5'-GTCTTACCTG**AAC**TCTGGAAT-3'
27	32M4			5'-TCTTACCTG**GCC**TCTGGAATC-3'
28	32M5			5'-TCTTACCTG**GTC**TCTGGAATC-3'
29	32M6			5'-TCTTACCTG**GGC**TCTGGAATC-3'
30	32M7			5'-CTTACCTG**GAG**TCTGGAATCC-3'
31	32M8			5'-CTTACCTG**GAT**TCTGGAATCC-3'
32	32M9			5'-CTTACCTG**GAA**TCTGGAATCC-3'
33	32D			5'-AGTCTTACCTGTCTGGAATCC-3'
34	33W		33	5'-TACCTGGACTCTGGAATCCAT-3'
35	33M1			5'-TTACCTGGAC**ACT**GGAATCCA-3'
36	33M2			5'-TTACCTGGAC**GCT**GGAATCCA-3'
37	33M3			5'-TTACCTGGAC**CCT**GGAATCCA-3'
38	33M4			5'-TACCTGGAC**TGT**GGAATCCAT-3'
39	33M5			5'-TACCTGGAC**TAT**GGAATCCAT-3'
40	33M6			5'-TACCTGGAC**TTT**GGAATCCAT-3'
41	33M7			5'-ACCTGGAC**TCA**GGAATCCATT-3'
42	33M8			5'-ACCTGGAC**TCG**GGAATCCATT-3'
43	33M9			5'-ACCTGGAC**TCC**GGAATCCATT-3'
44	33D			5'-TTACCTGGACGGAATCCATTC-3'

<Table 1c>

SEQ ID No.	Oligonucleotide	Exon	Codon	Sequence
45	34W	3	34	5'-CTGGACTCTGGAATCCATTCT-3'
46	34M1			5'-CCTGGACTCT**TGA**ATCCATTC-3'

47	34M2			5'-CCTGGACTCT**AGA**ATCCATTC-3'
48	34M3			5'-CCTGGACTCT**CGA**ATCCATTC-3'
49	34M4			5'-CTGGACTCT**GTA**ATCCATTCT-3'
50	34M5			5'-CTGGACTCT**GCA**ATCCATTCT-3'
51	34M6			5'-CTGGACTCT**GAA**ATCCATTCT-3'
52	34M7			5'-TGGACTCT**GGT**ATCCATTCTG-3'
53	34M8			5'-TGGACTCT**GGG**ATCCATTCTG-3'
54	34M9			5'-TGGACTCT**GGC**ATCCATTCTG-3'
55	34D			5'-CCTGGACTCTATCCATTCTGG-3'
56	35W		35	5'-GACTCTGGAATCCATTCTGGT-3'
57	35M1			5'-GGACTCTGGA**GTC**CATTCTGG-3'
58	35M2			5'-GGACTCTGGA**CTC**CATTCTGG-3'
59	35M3			5'-GGACTCTGGA**TTC**CATTCTGG-3'
60	35M4			5'-GACTCTGGA**ACC**CATTCTGGT-3'
61	35M5			5'-GACTCTGGA**AGC**CATTCTGGT-3'
62	35M6			5'-GACTCTGGA**AAC**CATTCTGGT-3'
63	35M7			5'-ACTCTGGA**ATG**CATTCTGGTG-3'
64	35M8			5'-ACTCTGGA**ATA**CATTCTGGTG-3'
65	35M9			5'-ACTCTGGA**ATT**CATTCTGGTG-3'
66	35D			5'-GGACTCTGGACATTCTGGTGC-3'

<Table 1d>

SEQ ID No.	Oligonucleotide	Exon	Codon	Sequence
67	37W	3	37	5'-GGAATCCATTCTGGTGCCACT-3'
68	37M1			5'-TGGAATCCAT**ACT**GGTGCCAC-3'
69	37M2			5'-TGGAATCCAT**CCT**GGTGCCAC-3'
70	37M3			5'-TGGAATCCAT**GCT**GGTGCCAC-3'
71	37M4			5'-GGAATCCAT**TAT**GGTGCCACT-3'
72	37M5			5'-GGAATCCAT**TGT**GGTGCCACT-3'
73	37M6			5'-GGAATCCAT**TTT**GGTGCCACT-3'
74	37M7			5'-GAATCCAT**TCA**GGTGCCACTA-3'
75	37M8			5'-GAATCCAT**TCG**GGTGCCACTA-3'
76	37M9			5'-GAATCCAT**TCC**GGTGCCACTA-3'
77	37D			5'-TGGAATCCATGGTGCCACTAC-3'
78	38W		38	5'-ATCCATTCTGGTGCCACTACC-3'
79	38M1			5'-AATCCATTCT**AGT**GCCACTAC-3'

80	38M2			5'-AATCCATTCT**CGT**GCCACTAC-3'
81	38M3			5'-AATCCATTCT**TGT**GCCACTAC-3'
82	38M4			5'-ATCCATTCT**GAT**GCCACTACC-3'
83	38M5			5'-ATCCATTCT**GCT**GCCACTACC-3'
84	38M6			5'-ATCCATTCT**GTT**GCCACTACC-3'
85	38M7			5'-TCCATTCT**GGA**GCCACTACCA-3'
86	38M8			5'-TCCATTCT**GGG**GCCACTACCA-3'
87	38M9			5'-TCCATTCT**GGC**GCCACTACCA-3'
88	38D			5'-AATCCATTCTGCCACTACCAC-3'

<Table 1e>

SEQ ID No.	Oligonucleotide	Exon	Codon	Sequence
89	41W	3	41	5'-GGTGCCACTACCACAGCTCCT-3'
90	41M1			5'-TGGTGCCACT**TCC**ACAGCTCC-3'
91	41M2			5'-TGGTGCCACT**GCC**ACAGCTCC-3'
92	41M3			5'-TGGTGCCACT**CCC**ACAGCTCC-3'
93	41M4			5'-GGTGCCACT**AGC**ACAGCTCCT-3'
94	41M5			5'-GGTGCCACT**ATC**ACAGCTCCT-3'
95	41M6			5'-GGTGCCACT**AAC**ACAGCTCCT-3'
96	41M7			5'-GTGCCACT**ACA**ACAGCTCCTT-3
97	41M8			5'-GTGCCACT**ACT**ACAGCTCCTT-3'
98	41M9			5'-GTGCCACT**ACG**ACAGCTCCTT-3'
99	41D			5'-TGGTGCCACTACAGCTCCTTC-3'
100	45W		45	5'-ACAGCTCCTTCTCTGAGTGGT-3'
101	45M1			5'-CACAGCTCCT**ACT**CTGAGTGG-3'
102	45M2			5'-CACAGCTCCT**GCT**CTGAGTGG-3'
103	45M3			5'-CACAGCTCCT**CCT**CTGAGTGG-3'
104	45M4			5'-ACAGCTCCT**TGT**CTGAGTGGT-3'
105	45M5			5'-ACAGCTCCT**TAT**CTGAGTGGT-3'
106	45M6			5'-ACAGCTCCT**TTT**CTGAGTGGT-3'
107	45M7			5'-CAGCTCCT**TCA**CTGAGTGGTA-3'
108	45M8			5'-CAGCTCCT**TCG**CTGAGTGGTA-3'
109	45M9			5'-CAGCTCCT**TCC**CTGAGTGGTA-3'
110	45D			5'-CCACAGCTCCTCTGAGTGGTA-3'

<Table 1f>

SEQ ID No.	Oligonucleotide	Exon	Codon	Sequence
111	48W	3	48	5'-TCTCTGAGTGGTAAAGGCAAT-3'
112	48M1			5'-TTCTCTGAGT**AGT**AAAGGCAA-3'
113	48M2			5'-TTCTCTGAGT**TGT**AAAGGCAA-3'
114	48M3			5'-TTCTCTGAGT**CGT**AAAGGCAA-3'
115	48M4			5'-TCTCTGAGT**GAT**AAAGGCAAT-3'
116	48M5			5'-TCTCTGAGT**GCT**AAAGGCAAT-3'
117	48M6			5'-TCTCTGAGT**GTT**AAAGGCAAT-3'
118	48M7			5'-CTCTGAGT**GGA**AAAGGCAATC-3'
119	48M8			5'-CTCTGAGT**GGC**AAAGGCAATC-3'
120	48M9			5'-CTCTGAGT**GGG**AAAGGCAATC-3'
121	48D			5'-TTCTCTGAGTAAAGGCAATCC-3'

Each oligonucleotide was mixed with a micro spotting solution (TeleChem International Inc, Sunnyvale, CA) at a mix ratio of 1:1, and 40 ㎕ of each oligonucleotide was transferred to a 96-well plate. Twenty pmol/㎕of oligonucleotides were spotted for codons 37, 41 and 45, and 40 pmol/㎕ for the remaining eight codons. After the charged 96-well plate was placed in a pin microarrayer (Microsys 5100 Cartesian, Cartesian Technologies Inc, Irvine, CA), each oligonucleotide was printed on an aldehyde-coated glass slide (26×76×1 ㎜, CEL Associates Inc, Houston, TX). Spots, each of 130 ㎛ diameter in size, were arranged in multiple columns and rows at intervals of 300 ㎛. The glass slide spotted with the oligonucleotides was washed twice with 0.2% SDS, and then, once with distilled water. The glass slide was soaked in hot water (95℃) to denature the oligonucleotides, and then, in 0.09 M sodium borohydride solution for 5 minutes to inactivate unreacted aldehyde groups. Then, the glass slide was washed twice with 0.2% SDS, and then, once with distilled water, centrifuged, and dried.

(Step 2) Preparation of DNA sample

Specimens of 74 colorectal carcinomas were collected from Seoul National University Hospital and 31 colorectal cancer cell lines were obtained from the Korean Cell Line Bank (KCLB). Of the 74 colorectal cancers, 34 were from the proximal colon (cecum to splenic flexure) and 40 were from the distal colorectum (splenic flexure to rectum). Of 31

colorectal cancer cell lines, 7 originated from the proximal colon and 6 from the distal colorectum. The origin of the remaining 18 colorectal cancer cell lines was unknown. The gastric cancer cell lines SNU-638 and SNU-719 were used as positive controls for β-catenin mutations (Woo, D. K. et al., *Int. J. Cancer* 95:108-113, 2001). SNU-638 has β-catenin mutation at codon 41 (ACC→GCC, Thr→Ala) and SNU-791 mutation at codon 34 (GGA→GTA, Gly→Val).

Genomic DNA was extracted from frozen specimens using TRI reagent (Molecular Research Center, Cincinnati, OH, USA) or the automatic magnetic bead-based system (KingFisher, ThermoLabsystems, Finland), following the manufacture's instructions. To generate a fluorescent dye-labeled DNA sample, PCR amplification was performed using the extracted DNA as a template and two pairs of primers described in SEQ ID Nos. 122 to 125 (MWG-Biotech, Ebersberg, Germany). As shown in table 2, PCR primers of SEQ ID Nos. 122 and 123 for exon 3 were used as described in Mirabelli-Primdahl, L. et al. (*Cancer Res.* 59:3346-51, 1999), and PCR primers of SEQ ID Nos. 124 and 125 for interstitial large deletion of β-catenin gene were used as described in Udatsu Y. et al. (*Pediatr. Surg. Int.* 17:508-512, 2001).

<Table 2>

SEQ ID No.	Primer	Amplified region	Amplified size	Sequence
122	Exon-3F	Exon 3	218 bp	5'-GATTTGATGGAGTTGGACATGG-3'
123	Exon-3R			5'-TGTTCTTGAGTGAAGGACTGAG-3'
124	Long-3F	Part of exon 2~ part of exon 3	1115 bp	5'-AAAATCCAGCGTGGACAATGG-3'
125	Long-3R			5'-TGTGGCAAGTTCTGCATCATC-3'

Each PCR reaction solution (25 ㎕) contained 100 ng of genomic DNA, 10 pmol of each primer, 40 μM of dCTP, 20 μM of fluorescent dye Cy5-dCTP (MEN) or Cy3-dCTP (Amersham-biotech Ltd., Buckinghamshire, UK). Reactions were initiated by denaturation for 5 min at 94℃ in a programmable thermal cycler (Perkin Elmer Cetus 9600; Roche Molecular Systems, Inc., NJ). PCR conditions consisted of 35 cycles of 30 sec at 94℃, 30 sec at 56℃, and 1 min at 72℃, with a final elongation of 7 min at 72℃. After the PCR amplification, Cy5- or Cy3-labeled PCR product was purified using a purification kit (Qiagen Inc, Valencia, CA) and digested

with 0.25 U of DNase I (Takara, Shiga, Japan) at 25℃ for 10 min. Remaining enzyme was inactivated at 85℃ for 10 min, removed by repeating the above purification procedure, and the Cy5- or Cy3-labeled DNA sample was recovered.

(Step 2) Hybridization reaction and analysis

The Cy5- or Cy3-labeled DNA samples prepared in step (1) were mixed and resuspended in 5× hybridization solution (TeleChem International Inc, Sunnyvale, CA) to a volume of 2~4 ㎕. Two ㎕ of the mixed DNA sample prepared in Example 1was dropped on the glass slide and the glass slide was covered with a cover glass. The hybridization reaction was performed by incubating the glass slide in a saturated vapor tube at 56℃ for 3 hours. The hybridized glass slide was rinsed at room temperature in a buffer of 0.2% SDS + 2× SSC for 15~30 min, and then, in distilled water for 5 min, followed by centrifuging and drying. The glass slide was scanned using a ScanArray Lite (Parkard Instrument Co, Meriden, CT) and analyzed using Imagine (Biodiscovery, version 4.2) and Quantitative Microarray analysis software (QuantArray, version 2.0).

Eleven wild type signals were compared to each other and adjusted to be equal by signal normalization. The remaining 110 signals at each codon were also adjusted in the same way as the wild type signals. After signal normalization, all signals were re-analyzed as previously described (Kim, I. J. et al., *Clin. Cancer Res.* 8:457-463, 2002). The mean (BA) and the standard deviation (BSD) of the background signals were calculated, and the cutoff level was established to be BA+2.58BSD. (BA+2.58BSD) indicated the upper limit of the 99% confidence interval, and signals over this value were identified as meaningful signals. All data analysis was carried out using a SigmaPlot (SPSS Inc., San Rafael, CA), and means and standard deviations were calculated using Microsoft Excel program. The results of mutational analyses of colorectal carcinomas and colorectal cancer cell lines are shown in Table 3.

<Table 3>

Sample		β-catenin mutation			MSI	APC mutation
Name	Type	Location	Codon	Mutation		
207	Tumor	Ascending[c]	32	GAC→AAC	+[f]	-[g]
395	Tumor	Ascending	45	TCT→TTT	+	-

396	Tumor	Ascending	45	In-frame deletion	+	-
400	Tumor	Ascending	45	TCT→TTT	+	-
435	Tumor	Ascending	41	ACC→GCC	+	-
SNU-407[a]	Cell line	Transverse[d]	41	ACC→GCC	+	-
SNU-1047[a]	Cell line	Transverse	45	TCT→TTT	+	4107delC
LS174T[b]	Cell line	Colon[e]	45	TCT→TTT	+	-
HCT116[b]	Cell line	Colon	45	In-frame deletion	+	-

[a] Oh, J. H. et al., *Int. J. Cancer* 81:902-910, 1999
[b] Ilyas, M. et al., *Proc. Natl. Acad. Sci. USA* 94:10330-10334, 1997
[c] Ascending colon
[d] Transverse colon
[e] Detailed information on the origin of these cell lines could not be found. It was confirmed that these cell lines originated from human colon adenocarcinomas.
[f] MSI in BAT-26
[g] No APC mutation was found in MCR

As shown in Table 3, mutations of the β-catenin gene were identified in 5 (tissue samples 207, 395, 396, 400 and 435) of 74 colorectal carcinomas (5/14, 7%). These five β-catenin mutations were identified in 34 proximal colon cancers (5/34, 15%) and none were found in 40 distal colorectal cancers (0/40, 0%). Of 34 proximal colon cancers, five β-catenin mutations were found in 25 right-sided colon cancers, and no mutation, in the 9 traverse colon cancers. These results suggest that β-catenin mutations are associated with the tumors in the proximal colon (p=0.017).

In 31 colorectal cancer cell lines, 4 β-catenin mutations were found in cell lines SNU-407, SNU-1047, LS174T and HCT116. Of these 4 β-catenin mutaions, two (SNU-407 and SNU-1047) were found in cell lines originating from the proximal colon (traverse colon). The origins of the other 2 cell lines (LS174T and HCT116) harboring β-catenin mutations were not determined.

A total of 9 mutations were found among 74 colorectal cancer tissues and 31 colorectal cancer cell lines. Eight mutations out of these 9

mutations were identified at GSK-3β phosphrylation sites. All point mutations were amino acid substitutions and occurred at codons 32, 41 and 45. Six mutations were concentrated at codon 45. Four of these 6 point mutations at codon 45 were the identical missense mutations (TCT→TTT, Ser→Phe; in samples 395, 400, SNU-1047 and LS174T) and the remaining 2 mutations, the same in-frame deletions as in samples 396 and HCT116. No interstitial large deletion of the β-catenin gene was detected.

In the case of tissue 400, an additional signal in combination with wild type signals was observed, which indicated a missense mutation at codon 45 (TCT→TTT, Ser→Phe) (Fig. 1). Eight of the 9 samples with β-catenin mutations showed both wild type signals at each codon and an aberrant signal, which indicated the presence of heterozygous mutation. Meanwhile, LS174T showed only an abnormal signal in the absence of a wild type signal at codon 45, which means that LS174T has homozygous β-catenin mutation.

All 9 samples with the β-catenin mutations were investigated for APC mutations in the MCR (codons 1263-1513). Only one cell line (SNU-1047) harbored APC truncation mutation at codon 1369 (4107delC). The cell line LS174T, which had been reported not to carry β-catenin mutation was found to harbor β-catenin mutation (codon 45, TCT→TTT, Ser→Phe) in the present invention (Ilyas, M. et al., *Proc. Natl. Acad. Sci. USA* 94:10330-10334, 1997).

<Example 2> Confirmation of β-catenin mutations detected by β-catenin oligo chip

In order to confirm β-catenin mutations detected by the inventive β-catenin oligo chip, the nine β-catenin mutation samples were subjected to PCR-SSCP, DHPLC, PTT, cloning sequencing and direct sequencing as follows.

PCR-SSCP and DHPLC analyses were performed as previously described (Kim, I. J. et al., *Int. J. Cancer* 86:529-532, 2000; Wagner, T. et al., *Genomics* 62:369-376, 1999). DHPLC analysis was done using WAVE (Transgenomic, Omaha, NE) and running conditions were optimized using WAVEMAKER software. A protein truncation test (PTT) was performed for mutation detection of the mutation cluster region (MCR, codon 1263-1513) of the *APC* gene, as previously described (Won, Y. J. et al., *J. Hum. Genet.* 44:103-108, 1999). During the cloning, fresh PCR products

were ligated into PCR-TOPO vectors, and subcloned using the TA cloning system (Invitrogen, Carlsbad, CA). Bi-directional sequencing was performed using a Taq dideoxy terminator cycle sequencing kit and an ABI 3100 DNA sequencer (Applied Biosystems, Forster City, CA).

<Table 4>

Sample	SSCP	DHPLC	Direct sequencing	Cloning sequencing	β-catenin oligo chip
207	+[a]	+	ND	+	+
395	+	+	+	+	+
396	+	+	+	+	+
400	ND[b]	+	ND	+	+
435	+	+	+	+	+
SNU-407	+	+	+	+	+
SNU-1047	+	+	+	+	+
LS174T	+	+	+	+	+
HCT116	+	+	+	+	+

[a] Detected
[b] Not detected

Among the conventional techniques, the automatic direct sequencing method, which has been widely used for mutational analysis, did not clearly detect 2 of the 9 β-catenin mutations (Fig. 2). PCR-SSCP also missed one β-catenin mutation (Fig. 3). These false-negative results might have been caused by excessive wild type DNA in cancer tissues or by the low sensitivity of these two methods.

<Example 3> Relationship between β-catenin mutations and MSI

It has been reported that MSI status can be meaningfully correlated with proximal colon cancers, and MSI may be used as a diagnostic marker for the diagnosis of proximal colon cancer (Traverso, G. et al., *Lancet.* 359:403-403, 2002). To determine the MSI status, genomic DNAs extracted from 74 colorectal carcinomas were subjected to PCR using BAT-26 marker (Shitoh, K. et al., *Genes Chromosomes Cancer* 30:32-37, 2001; Samowits, W. S. et al., *Am. J. Pathol.* 158:1517-1524, 2001).

Each PCR reaction solution (25 ㎕) contained 100 ng of genomic DNA extracted from normal and cancer tissues using Picoll-Paque and

Trizol reagents, 10 pmol of each BAT26-F and BAT26-R primers of SEQ ID Nos. 126 and 127 0.25 $\mu\ell$, 2.5 mM of dNTP 0.5 $\mu\ell$, 10×PCR buffer solution 2.5 $\mu\ell$, [α-^{32}P]dCTP 0.25 $\mu\ell$, and Taq DNA polymerase (5 unit/$\mu\ell$). Reactions were initiated by denaturation for 5 min at 94℃ in a programmable thermal cycler (Perkin Elmer Cetus 9600; Roche Molecular Systems, Inc., NJ). PCR conditions consisted of 35 cycles of 30 sec at 94℃, 30 sec at 52℃, and 1 min at 72℃, with a final elongation of 7 min at 72℃. The reaction mixture was heated at 95℃ for 5 min and cooled down in a ice bath. Thirty-five $\mu\ell$ of the cooled reaction mixture was subjected to 40% polyacrylamide gel (29:1) electrophoresis, and the gel was dried and exposed to X-ray.

To determine the correlations between the β-catenin mutations, MSI and tumor location, statistical analyses were performed using the χ^2 or Fisher's exact test, setting a=0.05 as the significance level using the STATISTICA software (StatSoft Inc., Tulsa, OK).

As a result, 12 of 74 colorectal cancer tissues (16%) showed MSI in the BAT-26 marker. 10 out of 34 proximal colon cancers (29%) were found to carry MSI and only 2 of 40 distal colorectal cancers (5%) were found to harbor MSI. MSI was statistically correlated with the proximal location ($p<0.01$). All 5 β-catenin mutations were found in 12 colorectal cancers with MSI (5/12, 42%) and none were found in 62 colorectal cancers with MSS (microsatellite stability). β-catenin mutations were more common in colorectal carcinomas with MSI than in those with MSS ($p<0.001$).

[EFFECT OF THE INVENTION]

As described above, the β-catenin oligo chip of the present invention employs the selectively designed oligonucleotides specific for the mutational hot spot areas of β-catenin gene, it provides improved accuracy and efficiency in detecting β-catenin gene mutation. Accordingly, the β-catenin oligo chip of the present invention can be used in studies to detect β-catenin mutations and unravel the signal transduction mechanism and tumorigenesis related to β-catenin gene.

What is claimed is:

1. A β-catenin oligonucleotide microchip for detecting β-catenin mutations comprising a plurality of oligonucleotides fixed on the surface of a solid matrix, wherein the oligonucleotides are designed to detect mutations at codons 29, 31, 32, 33, 34, 35, 37, 38, 41, 45 and 48 of β-catenin gene.

2. The β-catenin oligonucleotide microchip of claim 1, wherein the oligonucleotides are designed to detect 9 types of missense mutations, 1 type of in-frame deletion and a wild type at codons 29, 31, 32, 33, 34, 35, 37, 38, 41, 45 and 48.

3. The β-catenin oligonucleotide microchip of claim 2, which comprises the oligonucleotide of SEQ ID No. 1 for detecting wild type, the oligonucleotides of SEQ ID Nos. 2 to 10 for detecting missense mutations and the oligonucleotide of SEQ ID No. 11 for detecting in-frame deletions at codons 29; the oligonucleotide of SEQ ID No. 12 for detecting wild type, the oligonucleotides of SEQ ID Nos. 13 to 21 for detecting missense mutations and the oligonucleotide of SEQ ID No. 22 for detecting in-frame deletions at codons 31; the oligonucleotide of SEQ ID No. 23 for detecting wild type, the oligonucleotides of SEQ ID Nos. 24 to 32 for detecting missense mutations and the oligonucleotide of SEQ ID No. 33 for detecting in-frame deletions at codons 32; the oligonucleotide of SEQ ID No. 34 for detecting wild type, the oligonucleotides of SEQ ID Nos. 35 to 43 for detecting missense mutations and the oligonucleotide of SEQ ID No. 44 for detecting in-frame deletions at codons 33; the oligonucleotide of SEQ ID No. 45 for detecting wild type, the oligonucleotides of SEQ ID Nos. 46 to 54 for detecting missense mutations and the oligonucleotide of SEQ ID No. 55 for detecting in-frame deletions at codons 34; the oligonucleotide of SEQ ID No. 56 for detecting wild type, the oligonucleotides of SEQ ID Nos. 57 to 65 for detecting missense mutations and the oligonucleotide of SEQ ID No. 66 for detecting in-frame deletions at codons 35; the oligonucleotide of SEQ ID No. 67 for detecting wild type, the oligonucleotides of SEQ ID Nos. 68 to 76 for detecting missense mutations and the oligonucleotide of SEQ ID No. 77 for detecting in-frame deletions at codons 37; the oligonucleotide of SEQ ID No. 78 for detecting wild type, the oligonucleotides of SEQ ID Nos. 79 to 87 for detecting missense mutations and the oligonucleotide of SEQ ID No. 88 for detecting in-frame deletions at codons 38; the oligonucleotide of SEQ ID No.

89 for detecting wild type, the oligonucleotides of SEQ ID Nos. 90 to 98 for detecting missense mutations and the oligonucleotide of SEQ ID No. 99 for detecting in-frame deletions at codons 41; the oligonucleotide of SEQ ID No. 100 for detecting wild type, the oligonucleotides of SEQ ID Nos. 101 to 109 for detecting missense mutations and the oligonucleotide of SEQ ID No. 110 for detecting in-frame deletions at codons 45; and the oligonucleotide of SEQ ID No. 111 for detecting wild type, the oligonucleotides of SEQ ID Nos. 112 to 120 for detecting missense mutations and the oligonucleotide of SEQ ID No. 121 for detecting in-frame deletions at codons 48.

4. A manufacturing process of the β-catenin oligonucleotide microchip of claim 1, comprising

1) mixing each of the oligonucleotides in a micro spotting solution and distributing to a well plate;

2) spotting the oligonucleotides on the surface of a solid matrix using a microarrayer;

3) fixing the oligonucleotides on the solid matrix surface and washing;

4) denaturing the fixed oligonucleotides by soaking the solid matrix in 95□ water, and then, treating the solid matrix with a sodium borohydride solution; and

5) washing and drying the solid matrix.

5. The manufacturing process of claim 4, wherein each of the oligonucleotides used in step (1) has a 12 carbon spacer with 5' amino modification.

6. A method for detecting the β-catenin mutation using the β-catenin oligonucleotide microchip of claim 1, comprising

1) preparing a fluorescent dye-labeled DNA sample from the blood of a subject patient;

2) reacting the labeled DNA sample with oligonucleotide spots on the β-catenin oligo chip;

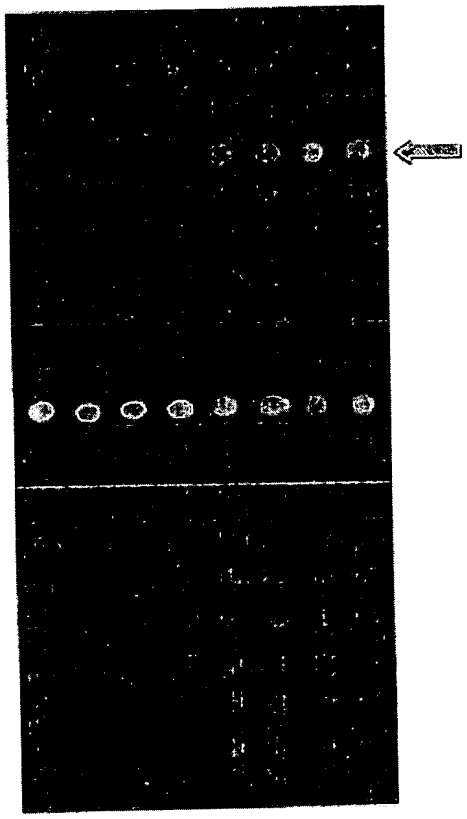
3) washing the reacted oligo chip to remove unbound sample DNA;

4) detecting the mode of hybridization of specific oligonucleotide spots using a fluorescence reader; and

5) examining the presence of gene mutation.

FIGURES

[Fig. 1]



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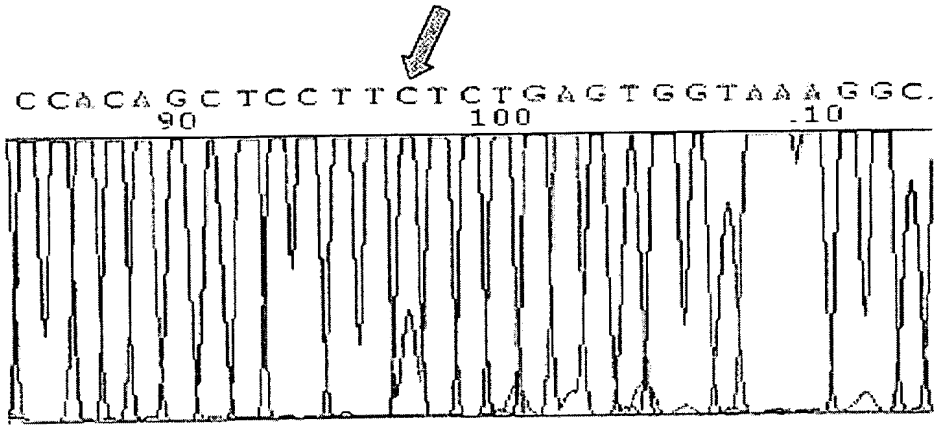
[Fig. 2]



[Fig. 3]